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Lycopene prevents experimental priapism against oxidative and nitrosative damage

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Abstract. – OBJECTIVE: Priapism is a persistent and often painful penile erection in the absence of sexual stimulation. It can cause progressive fibrosis, edema and drying of the erectile tissue and then it can lead to erectile dysfunction. Previous studies suggested that, neuronal nitric oxide levels increased during the priapism. High NO levels can result in the formation of reactive oxygen species (ROS) leading to oxidative stress in tissue and reproductive system. The aim of this study was to evaluate oxidative and nitrosative effects caused by pri in cavernosal tissue and serum, and deter beneficial effects of lycopene on ischemi apism.

MATERIALS AND METHODS: 32 rats w randomly divided into four gr the fi group being as the control. l se d group experimental ischemic pri n was med for an hour and then 1hour fusion rovid-ed. In the third grou yc a toneally given at th ose c g/kg. In the fourth group, lyc e were ad ered to rats with experimen sm.

RESULTS: apis used a sig ificant increase in ARS (thio ituric acid reactive substan and NO leve a significant decreas the le els of GSH, T, GPx and SOD in s n and rnosal tissue of rats. However, lyco si cantly increased GSH, CAT, GPx and S els bu creased formation of RS p ctio NO in rats with priapism.

CONCLU ur findings indicated that isch c priap lead to significant oxidative a sative damage in cavernosal tissue and es of rats. However lycopene treatment elimnates these negative effects induced priapism. For this reason, we suggested that pene may be used in the treatment of priapism.

Key Words:
Priapism, Lycopene, Oxidative stress, Nitrosative stress, Cavernosal tissue.

Introducti

Priapism a pe nt and often painful penile e in the a ce of sexual stimulation ough it has r occurred in domesti imals, it is a problem for human[3,4]. The eti- of priapis lated to use of some pharmac ical agen uch as antipsychotics and rec nal dr [-5]. Also it can be associated with cal disorders, metabolic disorders, trauma, tumors, neurological disorders and of the spider and scorpion[2,6]. Firstly, pria cause progressive fibrosis, edema and drying of the erectile tissue and then it can lead to erectile dysfunction[2]. Previous studies suggested that neuronal nitric oxide (NO) levels increased during the priapism[4,8]. Also, it was demonstrated that abnormally high NO levels can result in the formation of reactive oxygen species (ROS) leading to oxidative stress in tissue and reproductive system. This situation may cause severe infertility after priapism treatment. Therefore, it follows that antioxidant therapy may prevent side effects of priapism in the reproductive system.

Lycopene is a main carotenoid and is the most potent antioxidant among the various common carotenoids[9]. It is synthesized by microorganisms and plants red fruit and vegetables including tomatoes, watermelons, pink-grapefruits, apricots, papaya, guava and rosehip[10,11]. Humans and animals do not synthesize lycopene and, thus, depend on dietary sources mainly tomatoes[12]. Recently, many studies reported the beneficial effects of lycopene against cancer such as prostate and toxicities of antineoplastic agent (e.g. nephrotoxicity, hepatotoxicity) due to its highly efficient antioxidant and free radical scavenger properties[13-15]. The antioxidant activity of lycopene is assayed by inhibition

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of thiobarbituric acid reactive substances (TBARS) formation and induction of antioxidant defense system[16]. Atessahin et al[17] determined that lycopene prevents oxidative damage in testis tissue of rat. Similarly, many authors[12,13] claimed that prevention of oxidative stress by lycopene results in chemotherapeutic effect against many cancer types such as prostate. Since oxidative stress is one of the important results of priapism, it is, therefore, reasonable to consider that lycopene may play a role in the cellular defenses against oxidative stress induced by priapism.

Thus, in the current study, we aimed to determine of oxidative and nitrosative damage caused by ischemic priapism in serum and covernosal tissue and to find out whether or not lycopene has any beneficial effect against priapism in adult rats.

Materials and Methods

Chemicals

Lycopene 10% FS (Redivivo TM, Code 7803) was obtained from DSM Nutritional Products (Istanbul, Turkey). (Warwickshire, UK). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals

Thirty two healthy adult male Sprague-Dawley rats (between 2-3 months old and 250-300 g. in weight) obtained from the experimental Animal Institute, Malatya-Turkey were this study. Animals were kept in sterilized polypropylene rat cages, in 12-h light/dark cycle under an ambient temperature of 23°C. Diet and water for them were given *ad libitum*. All experiments were performed based on Animal Research Guidelines of Institutional Animals Ethics Committee.

Experimental Protocol

All operations were performed under sterile conditions. The animals were anesthetized with xylazine (10 mg/kg ip.) and ketamine injection (50 mg/kg ip) during the ischemic priapism and reperfusion period. Priapism was accomplished with the modified method described by Uluocak et al[18] and Sanli et al[19]. Briefly, a 50-cc syringe was used for the vacuum erection device and before application of vacuum to the penis, a constriction band, which was cut from 16 Fr Foley catheter in 2 mm slices, was loaded around the tip of the vacuum erection device. Then, the tip of the syringe was placed to the base of the penis and withdrawn gently to induce erection in the rat penis. After induction of erection in sufficient grade, the constriction band was placed to the base of the penis by slipping off the syringe (Figure 1).

The animals were divided into four groups with each containing eight rats. The first group was kept as the control group and given only distilled water as carrier. In the second group, ischemic priapism was formed during one hour. Then, the band was removed from the base of the penis and the penile tissue was allowed to reperfuse for one hour. In the third group, lycopene was administered intraperitonally (10 mg/kg) to rats without priapism. In the fourth group, lycopene was administered at 30th minute of priapism period and then reperfusion was provided for one hour in this group. End of reperfusion period, the animals were sacrificed and covernosal tissues were immediately removed and dissected over ice-cold glass. The homogenization of cavernosal tissues was carried out in a teflon-glass homogenizer with 1.15% mM KCl (pH 7.4) to obtain 1: 10 (w/v) dilution of the whole homogenate. Blood samples were collected from the left ventricle with an injector anesthesia. Sera were obtained by centrifugation (3000 g, 20 minutes, at 4°C) of whole blood. Tissue and serum samples were stored at –50°C in a deepfreeze until analysis.

Biochemical Assay

The levels of homogenized tissue and serum TBARS, as an index of lipid peroxidation, were determined by thiobarbituric acid reaction using the method of Yagi[20]. The product was evaluated spectrophotometrically at 532 nm and results are expressed as nmol/g tissue and nmol/ml. The reduced

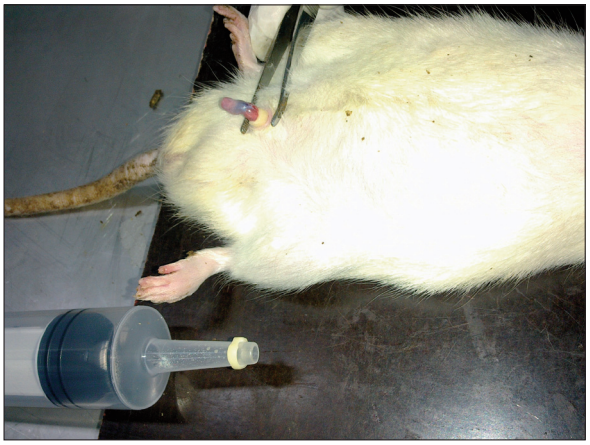

Figure 1. Formed experimental priapism models in rat by syringe.

glutathione (GSH) levels content of the testis homogenate was measured at 412 nm using the method of Sedlak and Lindsay[21]. The GSH level was expressed as nmol/ml. SOD (superoxide dismutase) activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2^- generated by the xanthine/xanthine oxidase system[22]. One unit of CuZn-SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate. The product was evaluated spectrophotometrically at 560 nm. Results are expressed as IU/mg protein. Catalase (CAT) activity of tissues was determined according to the method of Aebi[23]. The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. Tissue protein content was determined according to the method developed by Lowry et al using bovine serum albumin as standard[24].

Determination of NO

Serum nitric oxide (NO) level was determined by the evaluation of its stable oxidation products; nitrite (NO^-_2), and nitrate (NO^-_3). Serum n... levels were measured by the Griess rea... Reduction of nitrate to nitrite was accomp...d by catalytic reaction using cadmium. The ni... produced was determined by diazotization of s... fanilamide and coupling to na...ylene d... amine. Absorbance of this ...mple... as mea... sured at 545 nm. A sta... curve ...s estab... lished with a set of se...l d...ns ... trite. Linear regres... was ...by using the peak area from th... rite standa... he resulting equation was t... o calculate ...unknown sample con...ations. ...lts were expressed as moles pe... serum[26].

Sta...cal A...sis

T...eg... of significance was set at $p < 0.001$. ...way ana...s of variance (ANOVA) and post hoc Tukey-HSD test were used to determine the differences among the groups. All the analyses were carried out using the SPSS/PC (Version 11.5; SPSS, Chicago, IL, USA) software package program. Data are presented as mean±standard error of means (SEM).

Results

The cavernosal tissue S... GSH, ... nd TBARS levels are given ...able I. SOD, ... and GSH levels were ...nifica... decreas..., whereas TBARS l...ve... re... nificantly increased in rats wi...riapis... mpared ... control and other ...ps. There ... ...gnificant changes bet... control an... ene group. On the oth... hand, ...ycopene+priapism group SOD, CAT, GSH le... were increased, and TB...els were de...ed compared with th... priapism group. When given together with p...ism, lycope... brought SOD, CAT, GSH and T...RS levels cl... to the control level.

... SOD, ...ities and TBARS, GSH levels in ser... e given in Table II. In priapism group, a significant decrease in SOD activities ... GSH level was observed in the serum sam... ...rats compared with that of control and other groups. However, the TBARS and NO levels were significantly increased in the priapism group compared to other groups. In the lycopene+priapism group, TBARS, NO and GSH levels were near to control group value, but CAT and SOD activities remained unchanged compared with priapism group.

Discussion

Oxidative and/or nitrosative stress has an important role in the pathogenesis of ischemic priapism. It is largely known that the generation and

T... The SOD and CAT activities and GSH and TBARS levels in cavernosal tissue of rats.

	Control	Lycopene	Priapism	Priapism+ Lycopene	*p* value
T...ARS µmol/ml	3.92 ± 0.32[a]	2.91 ± 0.32[a]	5.86 ± 0.33[b]	2.92 ± 0.13[a]	0.001
GSH nmol/ml	109.5 ± 3.21[a]	114.3 ± 3.93[a]	92.2 ± 2.11[b]	111.3 ± 3.00[a]	0.001
SOD U/mg protein	16.24 ± 0.51[a]	16.55 ± 0.91[a]	12.16 ± 1.08[b]	13.91 ± 0.26[c]	0.005
CAT U/mg protein	0.053 ± 0.0014[a]	0.052 ± 0.0026[a]	0.028 ± 0.0016[b]	0.043 ± 0.0027[c]	0.001

Means bearing different superscripts within same row were significantly different.

Table II. The SOD and CAT activities and GSH and TBARS levels in serum samples of rats.

	Control	Lycopene	Priapism	Priapism+ Lycopene	*p* value
TBARS µmol/ml	3.33 ± 0.28[a]	3.06 ± 0.40[a]	6.63 ± 0.49[b]	4.20 ± 0.29[a]	0.001
GSH nmol/ml	66.14 ± 3.49[a]	63.46 ± 1.11[a,c]	40.63 ± 1.13[b]	57.01 ± 2.14[c]	
SOD U/mg protein	10.73 ± 0.61[a,c]	11.06 ± 1.51[a]	8.55 ± 0.42[b,c]	9.26 ±1.20[c]	
CAT U/mg protein	0.081 ± 0.0014[a]	0.084 ± 0.0026[a]	0.078 ± 0.0016[a]	0.083 ± 0.0027[a]	0.195
NO µmol/l	7.15 ± 0.88[a]	6.92 ± 0.51[a]	11.13 ± 0.31[b]	8.84 ± 0.42[c]	0.01

Means bearing different superscripts within same row were significantly different.

activity of reactive oxygen (ROS) and nitrogen species (RNS) in the penis influence vascular homeostasis of this organ, with adverse effects exerted at cellular and molecular levels[1,8]. In this context, we thought that ROS scavengers and antioxidant agents could be beneficial in the clinical management of ischemic priapism. In this work we showed that ischemic priapism lead to oxidative and nitrosative damage in both covernosal tissue and serum samples. On the other hand, lycopene treatment in ischemic priapism prevent oxidative and nitrosative stress and caused reoxygenation of the tissue.

TBARS, a degradation product from lip[illegible] droperoxidation, provides an index of perox[illegible]tion of lipids in biological tissues[27]. It has an important role in the etiology and pathogenesis several diseases and disorders [illegible] ischem priapism[2]. In our study the re[illegible] show that one hour priapism lead to a si[illegible]cant lip[illegible]eroxidation by increasing TB[illegible]RS [illegible]ls [illegible] and corporal tissue [illegible]ats. Si[illegible], Uluocak et al[18] determined th[illegible]e hour pr[illegible]m caused a significant inc[illegible]alondialde[illegible] level, a marker of oxidative str[illegible] rats. Besides, Kanika et al[2] als[illegible]served that [illegible]erimental priapism model[illegible] to damage in co[illegible]al tissue proteins via [illegible]eased l[illegible] peroxidations. Previous studies[illegible]firm[illegible] results. Lycopene could decrease elevate[illegible]RS lev[illegible]nduced by priapism and pre[illegible]lipi[illegible]roxidations. In agreement wi[illegible]ur fin[illegible]many previous studies clearly de[illegible]strated t[illegible]t lycopene is a potent antioxidant [illegible]id peroxidations[12,13,16].

SOD, CAT, GPx (enzymatic) and GSH (nonenzymatic) are members of body antioxidant defense [illegible]ms protecting against oxidative damage caused by free radicals production in normal physiological conditions and against molecules such as TBARS[28]. In the current study, it was shown that 1 hour priapism caused an attenuation in both enzymatic and nonenzymatic antioxidant defense systems via a decrease of [illegible], CAT[illegible]x and G[illegible] levels in rats. Howe[illegible]r, l[illegible]en[illegible]atment significantly induced a[illegible]dant[illegible]ity in [illegible]n and cavernosal ti[illegible]and coul[illegible]ve[illegible]xidative damage ca[illegible]iapism. T[illegible]esults were confirmed [illegible] previ[illegible]researches which showed that pri[illegible]m lead to a[illegible]ease of antioxidant capac[illegible]s[18]. Besides [illegible]al reports[29,30] clearly i[illegible]ated that the lycopene treatment increased antioxidant status i[illegible]any diseases including cancer a[illegible]oxication. [illegible] results clearly showed that 1 hou[illegible]pism [illegible]ed an imbalance in oxidant/antioxida[illegible]nd may lead to erectile dysfunctions due to cavernosal tissue damage in penis. [illegible]ver, lycopene can prevent oxidative damage [illegible]osal tissue and may reverse the deleterious effects of priapism.

NO, known as endothelium-derived relaxing factor (EDRF), is an important cellular signaling molecule involved in many physiological and pathological processes[31]. The endothelium of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increased blood flow[32-34]. Also, NO has an important function in penile erection via coordination with corpus cavernosal smooth muscle relaxation[32]. In our study it was observed that priapism caused a significant increase in NO levels, but lycopene decreased elevated NO levels in serum. Elevation of NO may be due to ischemia conditions and elevated oxidative stress in penis. Similarly, Uluocak et al[18] showed that priapism increase NO levels in serum and melatonin brought the levels of NO closer to normal levels. These findings agree with our findings.

Conclusions

Oxidative and nitrosative stress play important role in many ischemic disorders such as pri-

apism. For that, if oxidative and nitrosative stress are prevented with any agent, the damage caused by priapism can be reversed. In this study, it was observed that an hour priapism caused ischemia and a significant oxidative and nitrosative damage via an increase of TBARS and NO levels and a decrease of SOD, CAT, GSH and Gpx levels in serum and cavernosal tissue in rats. Additionaly, it was determined that lycopene eliminates oxidative and nitrosative damage in cavernosal tissue and serum due to their strong antioxidative properties. In conclusion because cavernosal damage may occur in erectile dysfunction in male, and therefore, quality of life may be impaired. lycopene may be used for the clinical treatment of priapism.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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